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Original Article

Rising Threat of Non-Fermenters in Respiratory Infections: Prevalence and Resistance Patterns in a Tertiary Care Hospital, India

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ABSTRACT

Background: Respiratory tract infections (RTIs) constitute a predominant group of infectious diseases worldwide. Unguarded and prophylactic use of antimicrobials has allowed nonfermenting gram-negative bacilli (NFGNB) to be considered important healthcare-associated pathogens.
Aims: To determine the prevalence and antimicrobial resistance patterns of non-fermenting gram-negative pathogens in respiratory infections.
Materials & Methods: All respiratory specimens (sputum or bronchoalveolar lavage fluid, endotracheal aspirate, and pleural fluid) were processed following standard microbiological procedures. After preliminary identification with automated VITEK System, susceptibility testing was performed with a VITEK-2 COMPACT system (BioMérieux, Marcy-l'Étoile, France).
Results & Conclusions: Our study found a high prevalence of nonfermenting Gram-negative bacteria (67%) compared to Enterobacterales (33%). Among the nonfermenters, Acinetobacter baumannii was the most common (56.4%), followed by Pseudomonas aeruginosa (35.6%), Burkholderia cepacia (1.98%), Sphingomonas paucimobilis (1.98%), Chryseobacterium indologenes (1.98%), and Stenotrophomonas maltophilia (1%). Carbapenems (76%), aminoglycosides (77%), cephalosporins (68%), and fluoroquinolones (55%) showed the highest resistance rates. Colistin (8.5%) and polymyxin B (4%) were the most effective antimicrobials. Nonfermenting Gram-negative bacteria (NFGNB) isolated from respiratory samples should not be dismissed as contaminants. Given the alarming rise in resistance, their clinical relevance must always be assessed in correlation with patient findings.

Keywords: *Respiratory tract Infections, Non-fermenters, Enterobacterales, antimicrobial resistance.*

INTRODUCTION

Respiratory tract infection is a prevalent infectious disease worldwide. In developing countries, it is the leading cause of morbidity and mortality in intensive care unit (ICU) patients. These patients are very closely associated with invasive devices and, therefore tend to be major reservoirs for hospital-acquired infections. Respiratory tract infections are usually

contracted through the air and by direct contact. Approximately 4.4% of patients admitted to the hospital have respiratory tract infections.[19] These infections, i.e., acute lower respiratory infections (ALRIs), constitute approximately 3–5% of deaths in India and 4.2 million deaths globally. [17] The etiological agents of RTI may vary with geography and time. Unguarded and prophylactic use of antimicrobials has allowed nonfermenting gram-negative bacilli (NFGNB) to be considered important healthcare-associated pathogens.[15]

Nonfermenter gram-negative bacilli can disseminate horizontally on fomites or the hands of healthcare workers.[16] Nonfermenting gram-negative bacilli (NFGNB) are a diverse group of bacilli that utilize oxygen aerobically. They are nonsporing bacilli that are not equipped to utilize glucose as an energy derivative despite being able to utilize it oxidatively rather than fermentatively. [1] Approximately 15% of the isolates from clinical samples are NFGNB. [11]

The majority of studies have focused on the identification of non fermenter gram-negative bacilli (NFGNB) in respiratory tract infections. In immunocompromised patients, the isolation of NFGNB strains and their antimicrobial susceptibility patterns have become imperative.

Aims & Objective:
The present retrospective study aims towards

a) To identify the prevalence of various NFGNBs isolated from respiratory samples
b) To understand their clinical relevance whether pathogen or contaminant
c) To analyze the antimicrobial resistance pattern

MATERIALS & METHODS:
This retrospective study was performed in the Department of Microbiology in a tertiary care center, from January 2024 to June 2024. All respiratory samples (sputum or bronchoalveolar lavage fluid, endotracheal aspirate, and pleural fluid) received during this period were included in the study. Our study was designed to identify and isolate NFGNB pathogens and, subsequently, their antimicrobial resistance patterns in various respiratory tract infections in our hospital.

Inclusion criteria:
Bacterial isolates with predominant growth isolated from different respiratory samples, sputum, endotracheal aspirates (ETA), bronchoalveolar lavage (BAL), and pleural fluid collected during the study period.

Exclusion criteria

a. All repeated bacterial culture isolates from previously reported patients
b. All commensal or contaminant bacterial isolates
c. Mixed bacterial growth (more than 3 types of bacterial isolates)
d. All pulmonary tuberculosis and fungal pneumonia patients were excluded from the study.

Processing, Isolation & Identification:
All respiratory samples (sputum, endotracheal aspirate, pleural fluid, and BAL) collected in the Department of Microbiology were processed, and the quality of each sample was assessed. Bartlett & American Society for Microbiology (ASM) grading was applied for all the sputum and endotracheal aspirates (ETA) for their quality check. [13, 9] As stated by Bartlett and ASM grading all sputum or ETA samples should have more than 25 leucocytes and fewer than 10 epithelial cells per low-power field of the microscope to obtain an acceptable and reliable specimen after Gram staining. Otherwise, samples would be rejected, and a repeat sample collection would be requested. All respiratory samples were directly inoculated onto blood agar, chocolate agar, and MacConkey's agar using a Nichrome wire loop. After 18–24 hours of incubation at 37°C, predominant growth was recorded. Bronchoalveolar lavage fluid was processed using quantitative culture, with a positive threshold of 10^4 CFU/mL.[4] Endotracheal aspirate samples were processed after 1 minute of vortexing at 3000 rpm for 10 minutes. All ETA samples were processed via semiquantitative culture via the calibrated loop method. A colony count of ≥105CFUs/mL signifies a potential pathogen.[13]

A total of 741 respiratory specimens, collected from 1st January to 30th June 2024, were included in the study. These methods include sputum (470), BAL (31), endotracheal aspirate (109), and pleural fluid (131). All samples were processed using standard microbiological procedures and incubated at 37°C for 18–20 hours. Growth was assessed semi-quantitatively for moderate to heavy growth and quantitatively when bacterial counts exceeded 10^5 CFU/mL. Preliminary identification was performed based on colony characteristics, morphology, hemolysis on blood agar, fermentation or non-fermentation, and oxidase reactions followed by Gram staining to confirm the presence of gram-positive or gram-negative cocci or bacilli. Pseudomonas aeruginosa, Burkholderia cepacia, Stenotrophomonas maltophilia (variable), Chryseobacterium indologenes, and Sphingomonas paucimobilis are oxidase-positive, glucose nonfermenting, gram-negative bacilli, and Acinetobacter baumannii are oxidase negative, nonfermenting, gram-negative bacilli; thus, the oxidase reaction is an important step toward the identification of NFGNB. [20] Thereafter, identification was performed with a VITEK-2 compact system (bioMérieux, Marcy-l'Étoile, France) using a Gram Negative (GN) card and Gram-Positive (GP) card according to the manufacturer's instructions. The system automatically inoculates the test card with the bacterial

sample, incubates it at a controlled temperature, and reads the colorimetric reactions at regular intervals.[8] After identification, antibiotic susceptibility testing was performed via an automated method, the VITEK-2 compact system, and Kirby–Bauer's disc diffusion method on Mueller–Hinton agar supplemented with 5% sheep blood, with the use of commercially available discs according to the Clinical Laboratory Standards Institute guidelines in some cases. [7]

Quality Control:

A standard operating protocol was followed for specimen collection; thereafter, the quality of the samples was assessed based on the American Society of Microbiology and Bartlett's acceptance and rejection criteria. Sterility control for the culture media was ensured by their quality control, with 5% of each batch being incubated at 37°C overnight. ATCC (American type culture collection) control strains, such as Escherichia coli (ATCC 25922), Staphylococcus aureus (ATCC 25923), and Pseudomonas aeruginosa (ATCC 27853), were tested on prepared media for quality control. For the antimicrobial susceptibility testing, a 0.5 McFarland standard was used to standardize the bacterial suspension density. [6]

RESULTS:

Prevalence of bacterial isolates:

Among the 741 samples collected during the study period from 1st January to 30th June 2024. A total of 150/741 (20%) culture-positive samples were found. A total of 101/150 (67%) isolates were identified as nonfermenter, remaining were Enterobacteriaceae and gram-positive organisms. Amid of non-fermenters, Acinetobacter baumannii (56.4%) was the predominant nonfermenter GNB, followed by Pseudomonas aeruginosa (35.6%), Burkholderia cepacia (1.98%), Sphingomonas paucimobilis (1.98%), Chryseobacterium indologenes (1.98%), and Stenotrophomonas maltophilia (1%). (Figure 1)

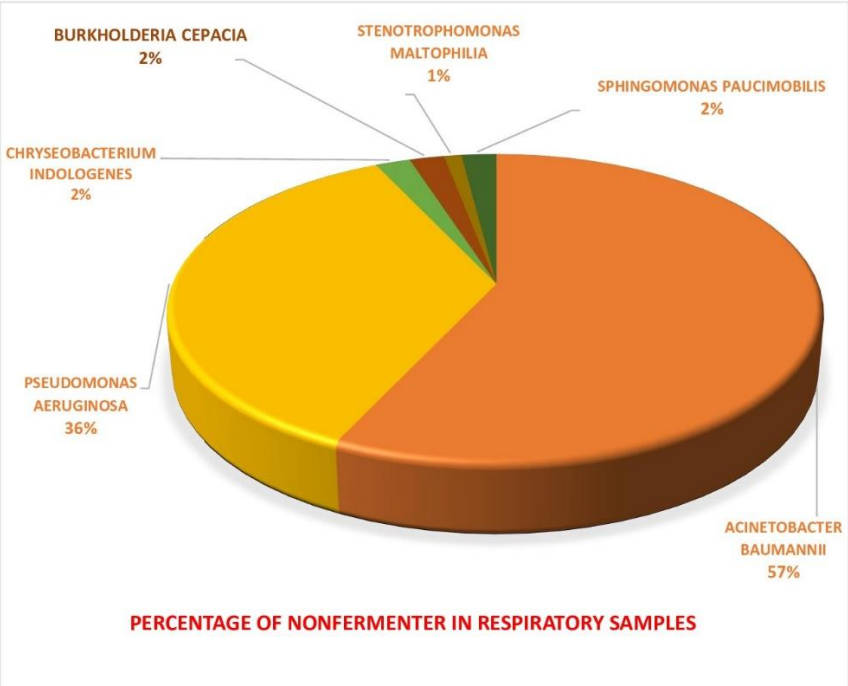

Figure 1: Percentages of nonfermenters in respiratory samples

Demographic Characteristics:

Among the demographic patterns, males accounted for the majority of infections caused by nonfermenting Gram-negative bacteria (73%). Patients aged 50–90 years were the most affected (60.3%), followed by those aged 18–50 years (39%). Overall, 93% of the patients were admitted to intensive care units (ICUs), while the remaining 7% were from other hospital wards.

Antimicrobial resistance pattern of non-fermenter gram-negative isolates:

The most effective antimicrobial agent for NF-GNB in our study was polymyxin B/colistin. The overall resistance of NFGNB to carbapenems (imipenem-78%, meropenem-74%) was highest, followed by aminoglycosides (amikacin-69%, gentamicin-86%), with significantly high resistance to first, second, and third-generation cephalosporins (cetriaxone-89%, cefepime-64%, ceftazidime-51%), piperacillin-tazobactam (62%), cefoperazone-sulbactam (53.25%), fluoroquinolones (ciprofloxacin-71.4%, levofloxacin-39%), minocycline (42%), monobactam (Aztreonam-27.5%), colistin (8.5%) and polymyxin B (4%). Individual antimicrobial resistance patterns are depicted through a bar diagram for Acinetobacter baumannii (Figure 2), Pseudomonas aeruginosa (Figure 3), other non-fermenter bacteria, Burkholderia cepacia, Chryseobacterium Indologenes, Sphingomonas paucimobilis and Stenotrophomonas maltophilia (Figure 4).

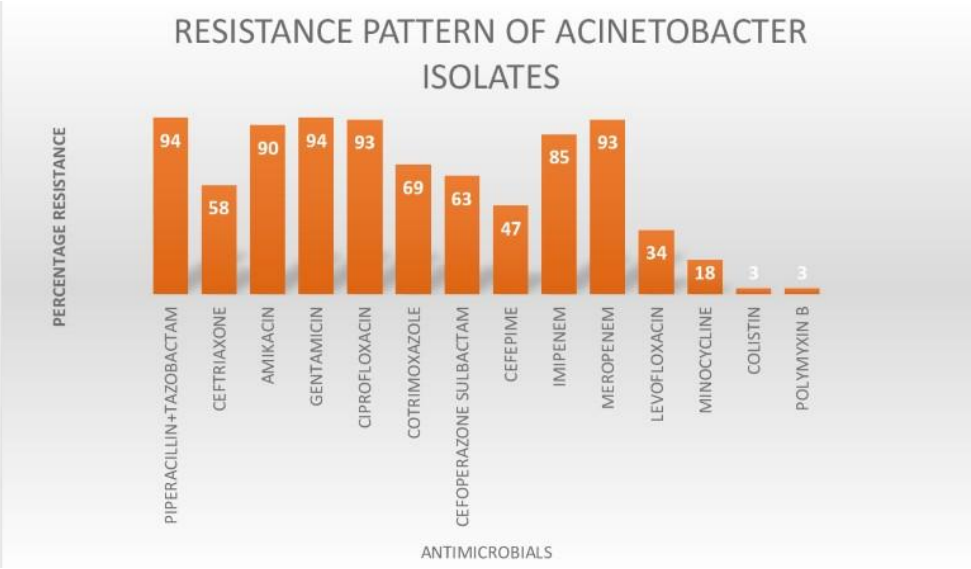

Figure 2 Antimicrobial resistance pattern of Acinetobacter baumannii in respiratory samples

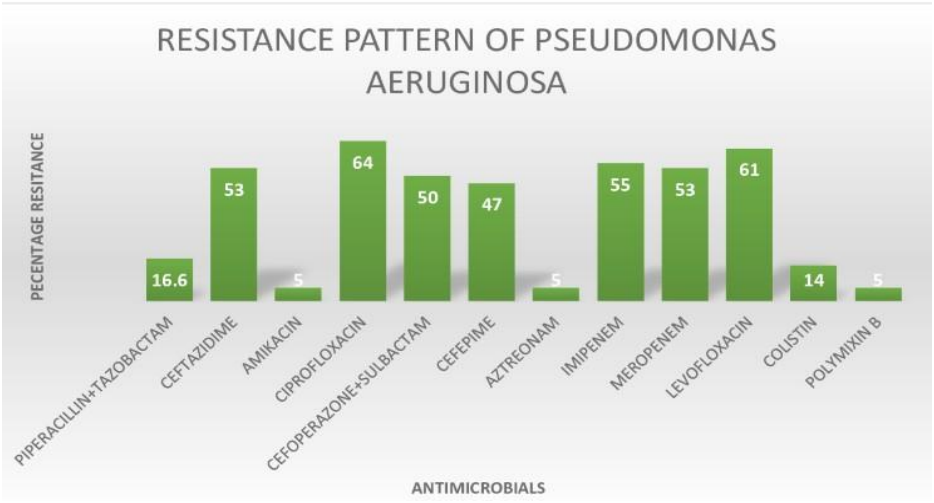

Figure 3 Antimicrobial resistance pattern of Pseudomonas aeruginosa in respiratory samples

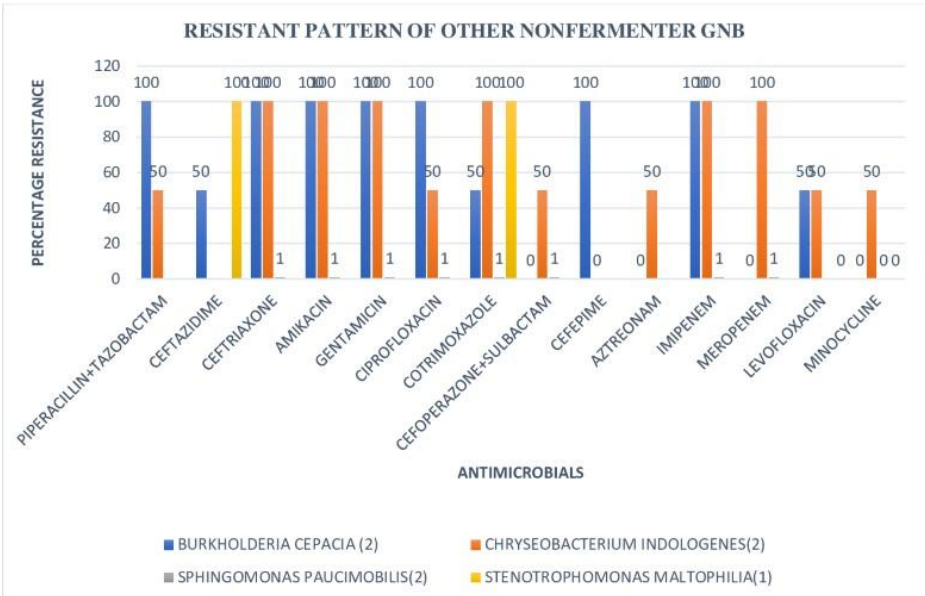

Figure:4 Antimicrobial resistance patterns of other nonfermenters (Burkholderia cepacia, Chryseobacterium indologenes, Sphingomonas paucimobilis, Stenotrophomonas maltophilia) and gram-negative bacilli in respiratory samples

Our study revealed that levofloxacin and minocycline are considered good options following colistin and polymyxin B for life-threatening respiratory infections, as these pathogens are very resistant. NFGNB presents a wide and inconsistent spectrum of antibiotic resistance. There were no such isolates found in the study for which all the antimicrobials were susceptible.

DISCUSSION:

Non-fermenting Gram-negative bacteria (NFGNB) have been implicated in causing respiratory infections, including pneumonia, cystic fibrosis, pleural infections, and various other lower respiratory tract infections. These infections are crucial causes of morbidity and mortality for all age groups, especially patients with prolonged ICU stays, ventilators, or immunocompromised patients.[22]

NFGNB isolates are now emerging and becoming notorious nosocomial pathogens causing hospital-acquired infections and opportunistic infections in immunocompromised patients. Resistance to many classes of antimicrobials has increased among these organisms due to widespread misuse and over-the-counter prescription of antimicrobials. [10]

In respiratory infections, there was an overall high prevalence of nonfermenter GNBs (67%) compared with other gram-negative organisms such as Enterobacterales and gram-positive organisms (33%) included in our study, which is in agreement with studies performed in Karnataka.[12] A study conducted in South India reported NFGNB isolation in 16.4% of respiratory samples, which is lower than the prevalence observed in our study.[14] The importance of the isolation of nonfermenters has increased over the last few years, and many reports match our findings in healthcare-associated infections caused by NFGNB. [15] Contrary to our findings, a study conducted in the Kolar district of India reported the isolation of NFGNB in 6.8% (25 out of 365) of respiratory samples. [14] Acinetobacter baumannii (56.4%) was the predominant non-fermenter isolate, followed by Pseudomonas aeruginosa (35.6%), Burkholderia cepacia (1.98%), Sphingomonas paucimobilis (1.98%), Chryseobacterium indologenes (1.98%) and Stenotrophomonas maltophilia (1%). This finding is consistent with other research conducted in Cuttack, Odisha, India.[21] Few studies from Madhya Pradesh, Eastern India, have aligned with the above findings from our research.[23, 18] Another study revealed that Acinetobacter baumannii was the primary etiological agent, accounting for 46.8% of respiratory tract infections, which is consistent with our findings.[3] In our study, carbapenem resistance was the highest at 76%, consistent with the results of a study conducted at a tertiary care center in Odisha.[2] Our study found a high level of resistance to cephalosporins (68%), which can be attributed to their widespread use, under- or overdosage, and incomplete or irregular treatment. These findings are consistent with those of Behera B et al., who reported a resistance rate of 82.18% to cephalosporins.[2] Aminoglycoside resistance was also greater than in other studies, but gentamicin resistance (86%) was greater than amikacin resistance (69%). [2] Beta-lactam–beta-lactam inhibitors (BLBIs) are resistant to approximately 52% and 62%, for piperacillin–tazobactam and cefoperazone–sulbactam, respectively. For the fluoroquinolone class of antimicrobials, the resistance rate was 71.4% for ciprofloxacin and 39% for levofloxacin, followed by the tetracycline group; we have reported resistance rates of up to 42% for minocycline. Even for the last resort of antibiotics, i.e., colistin and polymyxin b, the resistance rates were 8.5% and 4%, respectively, for those nonfermenters, who are not intrinsically resistant. This finding is consistent with the studies conducted by B. Behera et al., Chawla K, et al. 32 and Sharma S et al. 18, Soni M et al. [2, 5,21, 23]

Relatively high resistance rates have been observed for carbapenems, cephalosporins, beta-lactamase inhibitors, and fluoroquinolones in the context of NFGNBs. To overcome resistance, accurate bacteriological identification followed by antimicrobial susceptibility testing could help clinicians in prescribing the right antimicrobial agent for the effective management of LRTI and prevention of antimicrobial resistance in intensive care units (ICUs).

CONCLUSION:

In conclusion, identifying whether non-fermenting Gram-negative bacteria (NFGNB) are pathogens or colonizers is crucial, especially in respiratory samples, given rising resistance rates. Our study found the highest resistance to carbapenems and cephalosporins, while colistin and polymyxin B remained the most effective, though colistin resistance is emerging. Strict infection control and antibiotic stewardship are essential to limit NFGNB spread. Future research should focus on tracking resistance trends, exploring novel therapies, and correlating microbiological findings with patient outcomes to refine treatment strategies.

Limitations:

The limitations of this study encompass its single-center design, which may restrict the generalizability of the findings to other settings or regions. The investigation focused on specific respiratory specimens, such as sputum and bronchoalveolar lavage fluid, potentially overlooking other nonfermenting Gram-negative bacteria (NFGNB) infections. Furthermore, the absence of molecular data on resistance mechanisms constitutes a potential limitation that could influence the interpretation of the results.

Declarations;

Consent for publication: “Not Applicable” in this section

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Abbreviations:

- RTIs; Respiratory tract infections
- ALRIs; Acute lower respiratory infections
- NFGNB- Nonfermenter gram negative bacilli;
- BAL; Broncho alveolar lavage
- ETA; Endotracheal aspirate
- CFUs; Colony forming units
- ATCC; American type culture collection
- ICUs; Intensive care units

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